REMARKS

Claims 1-8 and 12-14 were originally filed with this application. Applicant has renumbered claims 12-14 as claims 9-11. Accordingly, the dependency of renumbered claim 9 has also been amended. Therefore, claims 1-11 are currently pending in this application. Claim 1 has been amended to more clearly recite that which applicants claims as their invention. This amendment contain no new matter, as support therefor is found in the specification at page 9, lines 15 to 29. A copy of the amendments made to the claims follow this preliminary amendment.

In addition, the specification has been amended to correct obvious typographical errors. In particular, the error at page 12, line 14, referring to "oligonucleotide 1 (SEQ ID NO:10)", is obvious in view of Table 1, at page 22, which shows that oligonucleotide 11 has the sequence set forth as SEQ ID NO: 10. Accordingly, no new matter has been introduced by way of these amendments. A copy of the amendments made to these paragraphs follows preliminary amendment.

Please charge our Deposit Account No. 08-0219 the basic Filing Fee of $355. The fee charged reflects the applicant's small entity status. In addition, please charge any fees or credit any overpayment to this account.

Respectfully submitted,

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Marked Up Version of Replacement Paragraphs in Specification Under 37 C.F.R. §1.121 (b)(1)(iii)

In the Specification:

Paragraph on page 5, lines 1-25:

Several preliminary studies on this topic have been published. Agrawal et al. *(Proc. Natl. Acad. Sci. (USA)* (1991) **88:**7595-7599) describes the [intravenously] intravenous and [intraperitoneally] intraperitoneal administration to mice of a 20mer phosphorothioate linked-oligonucleotide. In this study, approximately 30% of the administered dose was excreted in the urine over the first 24 hours with accumulation preferentially in the liver and kidney. Plasma half-lives ranged from about 1 hour ($t_{1/2\alpha}$) and 40 hours ($t_{1/2\beta}$), respectively. Similar results have been reported in subsequent studies (Iversen (1991) *Anti-Cancer Drug Design* **6:**531-538; Iversen (1994) *Antisense Res. Devel.* **4:**43-52; and Sands (1994) *Mol. Pharm.* **45:**932-943). However, stability problems may exist when oligonucleotides are administered intravenously and intraperitoneally. More recently, Agrawal et al. reported that oligonucleotide hybrids containing 2'-O-methyl ribonucleotides at both the 3'- and 5' ends and deoxyribonucleotide phosphorothioates in the interior portion were absorbed through the gastrointestinal (GI) tract of rats (*Biochem. Pharm.* (1995) **50**:571-576).

Paragraph at page 7, line 31 to page 8, line 12:

The term "non-phosphodiester-linkages" as used herein refers to a synthetic covalent attachment between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any number of chemical groups. Preferable synthetic linkages include alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, phosphoramidates, phosphoramidites, phosphate esters, carbamates, carbonates, phosphate

triesters, acetamidate, and carboxymethyl esters. In one preferred embodiment of the invention, [the] all of the nucleotides of the oligonucleotide [comprises] are linked via phosphorothioate and/or phosphorodithioate linkages.

Paragraph on page 9, lines 15-28:

For purposes of the invention, the term "2'-substituted oligonucleotide" refers to an oligonucleotide having a sugar attached to a chemical group other [that] than a hydroxyl group at its 2' position. The 2'-OH of the ribose molecule can be substituted with -O-lower alkyl containing 1-6 carbon atoms, aryl or substituted aryl or allyl having 2-6 carbon atoms, e.g., 2'-O-allyl, 2'-O-aryl, 2'-O-alkyl (such as a 2'-O-methyl), 2'-halo, or 2'-amino, but not with 2'-H, wherein allyl, aryl, or alkyl groups may be unsubstituted or substituted, e.g., with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl or amino groups.

Paragraph at page 9, line 30 to page 10, line 22:

In one preferred embodiment of the invention, the oligonucleotide administered includes at least one 2'-substituted ribonucleotide at its 3' terminus. In some embodiments, all but four or five nucleotides at its 5' terminus are 2'-substituted ribonucleotides, and in some embodiments, these four or five unsubstituted 5' nucleotides are deoxyribonucleotides. In other embodiments, the oligonucleotide has at least one 2'-substituted ribonucleotide at both its 3' and 5' termini, and in yet other embodiments, the oligonucleotide is composed of 2'-substituted ribonucleotides in all positions with the exception of at least four or five contiguous deoxyribonucleotide nucleotides in any interior position. Another aspect of

the invention includes the administration of an oligonucleotide composed of nucleotides that are all 2'-substituted ribonucleotides. Particular embodiments include oligonucleotides having a 2'-O-alkyl-ribonucleotide such as a [2'-0 methyl] 2'-O-methyl. Other embodiments include the administration of chimeric oligonucleotides. In one preferred embodiment, the chimeric oligonucleotide has at least one alkylphosphonate internucleotide linkage at both its 3' and 5' ends and having phosphorothioate internucleotide linkages.

Paragraph on page 11, lines 15-17:

In another embodiment, the oligonucleotide is complementary to a gene encoding a protein [in] associated with Alzheimer's disease.

Paragraph on page 12, lines 10-14:

FIG. 1 is a graphic representation showing the time course of radiolabelled oligonucleotide in liver, kidney and plasma following the oral administration of radiolabelled phosphorothioate (PS) oligonucleotide [1] 11 (SEQ ID NO:10);

Paragraph on page 19, lines 17-32:

The oligonucleotides administered to the animal may be hybrid oligonucleotides in that they contain both deoxyribonucleotides and at least one 2' substituted ribonucleotide. For purposes of the invention, the term "2'-substituted" means substitution at the 2' position of the ribose with, e.g., a -O-lower alkyl containing 1-6 carbon atoms, aryl or substituted aryl or allyl having 2-6 carbon atoms e.g., 2'-O-allyl, 2'-O-aryl, 2'-O-alkyl, 2'-halo, or 2'-amino, but not with 2'-H, wherein allyl, aryl, or alkyl groups may be unsubstituted or substituted, e.g., with halo, hydroxy, trifluoromethyl,

cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl or amino groups. Useful substituted ribonucleotides are [2'-0-alkyls] 2'-O-alkyls such as [2'-0-methyl] 2'-O-methyl.

Paragraph at page 35, line 32 to page 36, line 18:

The chemical form of radioactivity in rat plasma was further evaluated by HPLC as shown [is] in FIG. 4A and 4B, demonstrating the presence of both intact PS oligonucleotide (A) as well as metabolites (B) 12 hours after oral administration (see FIG. 4B). Intact oligonucleotide was also detected in rat liver 6 hours (FIG. 5B) and 12 hours (FIG. 5C) after oral administration. Radioactivity in rat brain, thymus, heart, lung, liver, kidney, adrenals, stomach, small intestine, large intestine, skeletal muscle, testes, thyroid, epidermis, whole eye, and bone marrow was detectable 48 hours after oral administration of the radiolabelled oligonucleotide. For unmodified oligonucleotide, minimal intact form was detectable in rat tissue samples. However, as shown in FIG. 11A for the hybrid oligonucleotide and in FIG. 11B for the chimeric oligonucleotide, intact oligonucleotides were detected in plasma and tissue samples of the liver, kidney, spleen, heart, and lung.

Paragraph on page 38, lines 20-26:

Oral absorption of oligonucleotides in fasting animals was also determined with PS- oligonucleotide and hybrid oligonucleotide. Decreased absorption rates were found, indicating that the retention time of the oligonucleotides in the gastrointestinal tract in the fasting animals may be lower [that] than in non-fasting animals.

Paragraph on page 39, lines 21-31:

An unmodified HIV-specific 25mer oligonucleotide and hybrid 25mer phosphorothioate-linked oligonucleotide having SEQ ID NO:10 and containing [2'-0-methyl] 2'-O-methyl ribonucleotide 3' and 5' sequences and a deoxyribonucleotide interior, as well as two hybrid 18mer phosphorothioate-linked oligonucleotides having SEQ ID NOS:20 and 21, and containing [2'-0-methyl] 2'-O-methyl ribonucleotide 3' and 5' sequences and a deoxyribonucleotide interior, were synthesized, purified, and analyzed as follows.

Amendments Made to the Claims

1.(Amended)A method for introducing an intact oligonucleotide into a mammal, the method comprising the step of orally administering to the mammal a chimeric oligonucleotide, the oligonucleotide comprising about 6 to 50 nucleotides linked via at least one phosphorothioate internucleotide linkage and at least one internucleotide linkage selected from the group consisting of alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, phosphoramidite, phosphate ester, carbamate, carbonate, phosphate triester, acetamidate, and carboxymethyl ester, the oligonucleotide further comprising at least one 2'-O-alkyl ribonucleotide, whereby the oligonucleotide is present in intact form in plasma at least six hours following oral administration.

9[12].(Amended)The method of claim [11] 1 wherein the oligonucleotide is complementary to a gene of a virus involved in a disease selected from the group consisting of AIDS, oral and genital herpes, papilloma warts, influenza, foot and mouth disease, yellow fever, chicken pox, shingles, adult T-cell leukemia, Burkitt's lymphoma, nasopharyngeal carcinoma, and hepatitis.

10[13].(Renumbered)The method of claim 1, wherein the oligonucleotide is complementary to a gene encoding a protein associated with Alzheimer's disease.

11[14].(Renumbered)The method of claim 1 wherein the oligonucleotide is complementary to a gene encoding a protein in a parasite causing a parasitic disease selected from the group consisting of amebiasis, Chagas' disease, toxoplasmosis, pneumocytosis, giardiasis, cryptoporidiosis, trichomoniasis, malaria, ascariasis, filariasis, trichinosis, schistosomiasis infections.